

USDA-ARS Western Regional Plant Introduction Station, Washington State University, Pullman, WA, USA

## Pathogenic Fungi in Garlic Seed Cloves from the United States and China, and Efficacy of Fungicides Against Pathogens in Garlic Germplasm in Washington State

F. M. DUGAN, B. C. HELLIER and S. L. LUPIEN

Authors' address: USDA-ARS Western Regional Plant Introduction Station, Washington State University, Pullman, WA 99164-6402, USA (correspondence to F. M. Dugan. E-mail: fdugan@wsu.edu)

Received November 20, 2006; accepted January 14, 2007

**Keywords:** *Allium sativum*, *Aspergillus*, benomyl, fludioxonil, *Embellisia*, *Fusarium*, *Penicillium*, thiophanate methyl

### Abstract

Commercially distributed garlic (*Allium sativum*) seed cloves from six states of the United States and mainland China were surveyed for the presence of fungi recorded as pathogenic to garlic in the literature. *Aspergillus niger*, *A. ochraceus*, *Botrytis porri*, *Embellisia allii*, *Fusarium oxysporum* f. sp. *cepae*, *F. proliferatum* and *Penicillium hirsutum*, were each recovered from one or more of these commercial sources, as was *F. verticillioides*, not previously reported as pathogenic to garlic, but here demonstrated to be a pathogen. Seed garlic distributed from public germplasm collections may also contain fungal pathogens: *E. allii*, *F. oxysporum* f. sp. *cepae* and/or *F. proliferatum* caused severe losses in 2002–2003 and 2005–2006 during germplasm regeneration and storage for the National Plant Germplasm System in Pullman, Washington. Use of fludioxonil, thiophanate methyl and/or benomyl (the latter withdrawn from the market, but used here as a standard) at label rates against *E. allii* and *Fusarium* species promoted plant health, but not when infections were located deep within tissues nor under some situations involving high disease pressure.

### Introduction

In 2002–2003, significant losses were documented during routine maintenance of the USDA-ARS National Plant Germplasm System (NPGS) clonal garlic (*Allium sativum* L.) collection in Pullman, Washington. Most losses appeared subsequent to harvest after detached bulbs were hanging in the drying shed prior to storage. Further losses from the same material were recorded after storage and just prior to fall planting of seed cloves. Significant losses again occurred in 2005–2006. The pathogens primarily responsible were *Fusarium proliferatum* (Matsushima) Nirenberg, recently reported on garlic in North America (Dugan et al., 2003), *Embellisia allii* (Campanile) Simmons and *Fusarium*

*oxysporum* Schlechtend.: Fr. f. sp. *cepae* (H. N. Hans.) W. C. Snyder & H. N. Hans.

Symptoms of rot from *Fusarium* spp. were frequently observed along the longitudinal axis (innermost leaf scales) of affected cloves from the 2002 harvest. Fungal growth could sometimes be discerned along this axis even when exterior tissues appeared healthy, an aetiology also reproduced in artificial inoculations (Dugan et al., 2003). In the 2003 harvest, symptoms were concentrated on bulb exteriors, probably because dry, hard soil was abrasive at lifting, presumably creating infection courts at harvest.

Because of our experiences with fungal infection of NPGS garlic germplasm, and because of analogous infection in some garlic obtained commercially for experimental purposes, we suspected that commercially distributed seed garlic might commonly contain miscellaneous fungi pathogenic to garlic. We resolved to survey commercially distributed seed garlic grown from various locations in the United States, and from mainland China, now a major exporter of garlic. We also conducted bioassays of our own field soil, to confirm presence of *Fusarium* pathogens in the soil.

Limited guidance is available for fungicides previously or currently labelled for garlic and potentially able to control *Fusarium* species. Crowe (1995) reported that benomyl is effective against *Fusarium culmorum* (W. G. Sm.) Saccardo, as well as *Penicillium*. Thiophanate methyl is labelled for *Penicillium* on garlic seed cloves in Washington State. Azoxystrobin fungicides are labelled for foliar diseases of garlic, but not for seed cloves in Washington State. Fludioxonil is labelled in Washington State for garlic, and according to the label controls *Fusarium* species, but it is not systemic.

There are occasional reports of chemicals (systemic or otherwise) used against *E. allii*. Portela et al. (1996) reported from Argentina that of thiram, quin-tozene (PCNB) and benomyl, the degree of control

was greatest with thiram and least with benomyl. An old experimental treatment in Italy used immersion of bulbs in 3% formalin before planting (Anon, 1925, citing Campanile, 1924). In Bulgaria, best results were with mercury chloride and thiram, followed by carb-oxin, chinisol, captafol, propineb, ziram, chlorothalonil, mancozeb and captan (Anon, 1980, citing Nakov et al., 1979). Depending on regulatory status, some of these applications (most notably mercury chloride and formalin) are no longer allowable.

In addition to conducting the survey of commercial seed garlic, we decided to test efficacies of thiophanate methyl and fludioxonil as preplanting dips for seed cloves, and to relate results to those obtained with benomyl, now withdrawn from the market, but previously found effective against *Fusarium* infections when used as a dip (Crowe, 1995). We also wished to test thiophanate methyl and to compare it to benomyl when these fungicides were used as postharvest dips. In each instance, our strategy was to conduct a minimum of one field experiment with natural levels of inoculum, plus one or more analogous experiments in the greenhouse or laboratory, in which inoculum levels could be precisely quantified. Manufacture of benomyl has been discontinued, with use allowed only under specified conditions (MacArthur, 2001; Damicone, 2002). Although application of thiophanate methyl on garlic replaces benomyl primarily as means to control *Penicillium* in seed cloves, it has the potential to reduce losses from *Fusarium* (e.g., Nan, 1995). Our objectives focus on germplasm management. Cost-effective treatment of bulbs destined for consumption is not addressed.

## Materials and Methods

### Survey of marketed seed garlic for fungal pathogens

Seed garlic cloves grown in California, Idaho, Oregon, New York, North Dakota, Washington State and People's Republic of China (province unknown) were purchased from commercial distributors in spring 2005. From each location, 25 bulbs were individually loosely wrapped in aluminium foil, placed inside a perforated plastic box and incubated at 28°C until symptoms of rot appeared. Tissues excised from margins of rotted areas were surface-disinfested for 2–3 min in 0.5% NaOCl with a distilled water rinse, and transferred to half strength (½) V8 agar (Ste-

vens, 1981) amended with 50 µg/ml streptomycin sulphate and 50 µg/ml tetracycline hydrochloride for recovery of fungi. Fungi were maintained on slants of 1/2V8 agar, stored in glycerol at –80°C and identified as described below.

### Isolation of fungi from USDA germplasm

In addition to fungi isolated as described above, fungi were recovered by closely analogous procedures from symptomatic garlic bulbs produced on the USDA Western Regional Plant Introduction Station (WRPIS) farm in 2001, 2002–2003 and 2005–2006. Fungi were stored in glycerol at –80°C or in liquid nitrogen vapour. *Fusarium proliferatum* was isolated, identified and reported for the first time as a cause of bulb rot of garlic in North American (Dugan et al., 2003).

### Confirmation of pathogenicity

To verify pathogenicity of taxa identified as pathogens of garlic in the literature and/or isolated by us (Table 1), we selected a minimum of one (but usually two or more) isolates of a given taxon from each source of seed garlic in which it was detected, and conducted artificial inoculations. Five to six cloves, disinfested in 0.5% NaOCl for 45 s, rinsed with sterile water, were injured to a depth of 4.5 mm with a probe of 1 mm diameter, and the wound filled with ½V8 agar colonized by the isolate. Controls used sterile agar in place of colonized agar. Cloves were placed individually in single wells of 6-well plates, and incubated on the laboratory bench top at 25–28°C for 3½–5 weeks for symptom development. We also tested two isolates of *Fusarium verticillioides* (Sacc.) Nirenberg, a species which, to our knowledge, has not been described as a pathogen of garlic, but which was repeatedly isolated by us. That the fungi so inoculated were responsible for the observed pathogenesis was confirmed by examination of sporulation within the lesions or, especially for *F. verticillioides*, by re-isolation back into pure culture.

### Identification of pathogens

Some *Fusarium* isolates recovered from garlic rotting in the field or drying shed were provisionally identified as *F. oxysporum* f. sp. *cepae* and tested by inoculation of representative isolate PI540346#3 into onion (*A. cepa* L.) bulbs and garlic cloves, using FOC-8

Table 1  
Fungi pathogenic to garlic and isolated from commercial seed garlic

Geographic source	Cultivar	Pathogenic fungi
California	Silver Rose, softneck	<i>Aspergillus niger</i> , <i>Embellisia allii</i> , <i>F. oxysporum</i> f. sp. <i>cepae</i> , <i>Fusarium proliferatum</i>
Idaho	Inchelium Red, softneck	<i>Botrytis porri</i> , <i>E. allii</i> , <i>Penicillium hirsutum</i>
Oregon	Siberian, hardneck	<i>A. ochraceus</i> , <i>F. proliferatum</i> , <i>P. hirsutum</i>
New York	Music, hardneck	<i>E. allii</i> , <i>F. oxysporum</i> f. sp. <i>cepae</i> , <i>F. verticillioides</i> <sup>a</sup> , <i>P. hirsutum</i>
North Dakota	Montana Roja, hardneck	<i>E. allii</i> , <i>F. oxysporum</i> f. sp. <i>cepae</i> , <i>F. proliferatum</i> , <i>P. hirsutum</i>
Washington State	Russian Giant, hardneck	<i>A. ochraceus</i> , <i>E. allii</i> , <i>F. oxysporum</i> f. sp. <i>cepae</i> , <i>P. hirsutum</i>
China	Spanish Roja, hardneck	<i>F. proliferatum</i> , <i>F. verticillioides</i> <sup>a</sup> , <i>P. hirsutum</i>

<sup>a</sup>*F. verticillioides* from China lost some aggressiveness in consecutive trials, but *F. verticillioides* from New York remained aggressive.

[*F. oxysporum* f. sp. *cepae* from Howard Schwartz (Swift et al., 2002)] as a positive control. Inoculum was produced on ½V8 agar, adjusted to ca.  $10^6$  conidia per ml and 25 µl of spore suspension were injected to each test bulb or clove with a sterile syringe. In each of the two replications for each host, five cloves or five bulbs were inoculated with the test isolate, five with the positive control and five with sterile water. Incubation conditions for inoculated materials followed Rengwalska and Simon (1986) with slight modification. *Botrytis* isolates were identified to species according to Chilvers and du Toit (2006), Ellis (1971), Presly (1985) and/or Yohalem et al. (2003). *Fusarium* isolates were identified to species with Leslie and Summerell (2006), Nelson et al. (1983) or Nirenberg and O'Donnell (1998). Isolates of *E. allii* were compared with descriptions in Corlett (1996), David (1991) and the key and descriptions in Simmons (1971, 1983). *Aspergillus* isolates were identified according to Klich (2002), and *Penicillium* isolates according to Centraalbureau voor Schimmelcultures, n.d., Frisvad and Samson (2004), Pitt (2000) and/or Samson et al. (2000).

#### Field trial of fungicides as preplanting dips

On 21 October 2003, 216 cloves of white-skinned, soft-necked garlic (*A. sativum*, 'California late variety') were planted at the WRPIS Pullman farm. Symptoms of bulb rot had been strongly evident in garlic grown in this field in 2002. We used 18 cloves per replication, with three replications for each of the three treatments and a control. Each treatment and a control were distributed at random in each of the three rows. Each row was separated from adjoining rows by a buffer strip. Treatments consisted of the control (no dip), or of preplanting dips of seed cloves with Maxim 4FS (fludioxonil 40.3% a.i.; Syngenta, Greensboro, North Carolina), Topsin M WSB (thiophanate-methyl dimethyl [(1,2-phenylene)-bis(iminocarbonothioyl)]bis [carbamate] 70% a.i.; Cerexagri, King of Prussia, Pennsylvania), or Benlate ('benomyl') (benomyl methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, 50% a.i.; DuPont, Wilmington, DE, USA) dipped as directed by the label, or for benomyl, as documented in Thompson (1991): Maxim, 52.5 µl in 50 ml dH<sub>2</sub>O per 54 cloves; Topsin 1.18 g/l; Benlate 1.18 g/l. Bulbs were lifted on 7 July 2004, hung in a drying shed (18–29°C, 35–55% RH) then cleaned on 10 September and stored again in the shed until bulbs were individually inspected and weighed on 21 October. Bulbs were randomized throughout storage.

#### Greenhouse trial of fungicides as preplanting dips

On 31 December 2003, 192 cloves of white-skinned, soft-necked garlic (same source as the above field trial) were planted into containers of potting mix (sterile Sunshine® potting soil + pasteurized field soil, 5 : 9 by volume, @ one clove per container) amended with inoculum of *F. proliferatum*, *F. oxysporum* f. sp. *cepae* or *E. allii*, or un-amended (for controls). Isolates used

were single spore isolates of *F. proliferatum* AsatF8 (Dugan et al., 2003), *F. oxysporum* f. sp. *cepae* PI540346#3 and *E. allii* PI615415#1a (the latter isolated from a rotted garlic bulb in Pullman in 2002), all maintained in the culture collection of WRPIS. Preparation of inoculum followed Park et al. (1988) modified by use of Palouse field soil and inoculation of soil with colonized-agar plugs, with final concentration in potting mix of ca.  $1.5 \times 10^3$  cfu/g for the *Fusarium* spp. and 500 cfu/g for *E. allii*. Prior to planting, cloves were either treated with fludioxonil, thiophanate methyl or benomyl as described above, or were untreated (for controls). Individual treatments consisted of two replications of six cloves each, such that each treatment utilized one of three fungicides and one of three pathogens. Fungicides and pathogens were individually paired in all possible combinations. Three sets of controls consisted of: (i) cloves dipped in fungicide and planted to un-amended potting mix, (ii) cloves not receiving fungicidal dip and planted to potting mix amended with a pathogen or (iii) cloves exposed to neither a fungicide nor pathogens. Plants were grown under natural and artificial light (high-pressure sodium and metal halide 1000 W, 12 h/day) until July 6, when plants were harvested and were scored by dry weight in g per living plant (dead plants received a score of zero).

#### Postharvest dips with thiophanate methyl or benomyl

On 19 August 2004, 12–15 plants were harvested from each of the 11 rows of red hard-necked garlic (W6-12839) growing on the WRPIS Pullman farm, stored overnight in a drying shed, then cleaned and subjected to one of the three treatments, using four to five plants from each row: postharvest dip with thiophanate methyl (1.18 g/l = 0.83 g active ingredient per l) or postharvest dip with benomyl (2.0 g/l = 1.0 g active ingredient per l) with dip times of 30 min at 28–29°C with mild agitation or a 'no dip' control. After treatment, bulbs were stored in the drying shed (conditions as above) until 12 December, when they were removed to the garlic storage room (10°C, 55% RH) until 1 March 2005, when bulbs were weighed, broken into component cloves and the number of rotted and healthy cloves recorded for each bulb. For analysis, treatments were blocked by row number.

#### Trials for eradication of pathogens in wounded and inoculated garlic bulbs

We conducted two trials which mimicked postharvest dips of material harvested from the field, but in which inoculum concentration was precisely quantified. Single spore isolates PI 540346#3-sci1, *Fusarium oxysporum* f. sp. *cepae*, and Asat F8, *Fusarium proliferatum*, were retrieved from liquid nitrogen vapour and transferred onto ½V8, and incubated at ambient laboratory temperature (ca. 22–25°C) under 12 h/day combined cool white and near ultraviolet lights. Conidia were harvested by washing the agar surface with sterile de-ionized water and adjusted to  $10^6$  conidia per ml.

Garlic bulbs harvested from two different locations in the fall of 2005 were employed. Accession W612839 'Gourmet Red' grown on the Plant Introduction farm in Pullman was inoculated with *F. oxysporum* f. sp. *cepae*, and 'German Red' from Potlatch, ID was inoculated with *F. proliferatum* as follows: bulbs were disinfested by being sprayed to runoff with 70% ethanol and allowed to dry in a biological safety cabinet, then wounded multiple times by being rolled once along the surface of a flame-disinfested wood rasp whose teeth were on a 2 mm × 3 mm grid and projected 1 mm. Pressure exerted was just sufficient to break clove skins. Wounded bulbs were inoculated by immersion in conidial suspensions for 10 s, allowed to drain and placed in paper bags for 12 h. In each of the two experiments (one with *F. oxysporum* f. sp. *cepae*, the other with *F. proliferatum*), three replications of five asymptomatic bulbs each were treated with dips of either thiophanate methyl, benomyl or not dipped. Wounding and inoculation were performed on 19 November 2005 and treatment with fungicide on 20 November 2005. Fungicide dips were maintained as slurries at 28–30°C at rates of 1.8 g/l for thiophanate methyl and 2.0 g/l for benomyl with dip times of 30 min. Bulbs were dried for 1 h, bagged by replication, randomized and placed in an incubator at 14°C, the temperature at which garlic germplasm is routinely stored at WRPIS.

#### Field soil assay

On 26 September 2002, soil was extracted with a clean shovel (disinfested between locations with ca. 10% household bleach) from five locations within a field of the WRPIS farm in which the garlic harvest of that year had been most severely impacted by bulb rots. Soil was loaded into clean buckets (disinfested as above) and the buckets were labelled by field, row and plot number and stored at –20°C until 14 January 2003 when soil from each of the five locations was dispensed into five clean pots (disinfested as above). Seeds of Spanish onion (*Allium cepa*, variety unknown), disinfested for 15 s in 70% ethanol followed by 5 min in 0.5% NaOCl and a sterile distilled water rinse, were germinated with sterile distilled water in autoclaved vermiculite. Four transplants were transferred to each of the five pots per location, each pot containing ca. 3.5 l of soil. For each location, one of the five pots of soil was autoclaved prior to planting, and served as a control. Plants were grown under natural and artificial light as above until 10 October 2003, when plants were lifted, bulbs examined and symptoms recorded; then bulbs were detached, labelled, randomized and stored at 28°C until 31 October, when symptoms were again recorded, and tissue from basal plates was excised, disinfested in 0.5% NaOCl for 2 min followed by dH<sub>2</sub>O rinse, transferred to ½V8 amended with antibiotics for suppression of bacteria and incubated at ca. 22°C for recovery of fungi. All *Fusarium* isolates recovered were streaked to produce single spore colonies, and grown on ½V8 for pathogenicity

tests: From each isolate, an agar plug of 3 mm diameter was inoculated into a wound on each side of two yellow sweet onions (*A. cepa*, variety unknown) to produce a total of four wounds. The process was replicated once for each isolate, and the onions stored for 7 days at ca. 22°C followed by 8 days at 28°C. At 15 days after inoculation, symptoms were recorded. Isolates inducing lesions were re-isolated and identified according to Nelson et al. (1983), or Nirenberg and O'Donnell (1998).

#### Statistical analysis

Data from fungicide experiments were subjected to PROC ANOVA and/or PROC GLM in SAS 8.2 (SAS Institute, Inc., Cary, NC, USA) and/or ANOVA in SYSTAT 9.0 (SPSS Science, Chicago, IL, USA).

#### Results

##### Survey of marketed seed garlic for fungal pathogens

All lots of commercial seed garlic contained species of fungi documented as pathogenic to garlic in published literature (Table 1). Although incubation conditions were the same for each lot, lots were not received nor processed simultaneously. No comparisons of pathogen frequency between lots are presented, but presence of pathogens is tabulated for each lot.

##### Confirmation of pathogenicity

Isolated from one or more sources (Table 1) and confirmed as pathogenic in our experimental inoculations were *Embellisia allii*, *Fusarium oxysporum* f. sp. *cepae*, *Fusarium proliferatum*, *Penicillium hirsutum* Dierckx and *Botrytis porri* Buchw. *Aspergillus ochraceus* K. Wilh. (= *A. alutaceus* Berk. & Curt.), reported pathogenic by Gargi and Roy (1988), was not consistently aggressive and sometimes did not differ from controls. *Aspergillus niger* Tiegh., listed as pathogenic by Sumner (1995), induced small lesions only rarely larger than discoloured areas in controls. *Embellisia allii* (usually only responsible for cosmetic damage, but capable of forming cankers) was not consistently aggressive, but sometimes formed small lesions up to 1 cm in diameter. *Fusarium verticillioides* MUS1B-2-sci1 from NY and SpR4A-1-sci2 from China induced discolouration and shrivelling on cloves, and were re-isolated into culture; when tests were repeated with garlic cloves, only MUS1B-2-sci1 again proved pathogenic. On onion, MUS1B-2-sci1 again proved pathogenic, but SpR4A-1-sci2 did not consistently differ from controls.

##### Isolation of fungi from USDA germplasm

Based on isolation frequency in 2002, *E. allii*, *F. proliferatum* and *F. oxysporum* f. sp. *cepae* accounted for approximately 11, 30 and 55% of infections, respectively. *Penicillium* spp. plus *Botrytis porri* accounted for approximately 4% of infections in 2002. *Embellisia allii*, *F. proliferatum* and *F. oxysporum* f. sp. *cepae* were also isolated from WRPIS garlic germplasm in subsequent seasons.

### Identification of pathogens

Table 1 lists pathogens identified from commercially distributed seed garlic. In addition, *Fusarium oxysporum* f. sp. *cepae*, morphologically identical to saprophytic *F. oxysporum*, was confirmed as one of the major pathogens at the USDA farm by the following results.

### Tests with onion

Lesions were produced on all 10 onion bulbs (five in rep 1 + five in rep 2) inoculated with the Pullman isolate PI540346#3, and these lesions were indistinguishable from lesions of the positive controls inoculated with the *F. o. f. sp. cepae* FOC8-sci3. All lesions produced by the Pullman isolate yielded fungi indistinguishable from the parent isolate upon plating to agar. No lesions were produced in controls inoculated with sterile water.

### Tests with garlic

Lesions were produced on all 10 garlic cloves (five each from reps 1 and 2) inoculated with Pullman isolate PI540346#3. When plated to agar media, all tissues excised from lesions yielded fungi indistinguishable from parent isolate PI540346#3. No lesions were detected on controls, nor were any fungi recovered from controls when tissue was excised from inoculation points and plated to agar. FOC8-sci3 was more aggressive than PI540346#3 on garlic. All cloves inoculated with FOC8-sci3 were completely rotted internally, whereas in cloves inoculated with the Pullman isolate lesions ranged from approximately 8 mm to the length of the clove. The other major pathogens recovered at the USDA farm were *F. proliferatum* (Dugan et al., 2003) and *E. allii*.

### Field trial of fungicides as preplanting dips

Effects of treatment were significant ( $P = 0.04$ ), but effects of replication and interaction were not significant ( $P = 0.31$ ,  $P = 0.72$ ), so replications were pooled for analysis. Preplanting application of fungicides to planting cloves resulted in higher mean bulb weights relative to controls for each fungicide, but only in the instances of fludioxonil and thiophanate methyl were results significant at conventional levels ( $P \leq 0.05$  and  $P \leq 0.10$ , respectively, Table 2).

Table 2  
Results of field trial, fungicides as preplanting dips for garlic cloves

Treatment <sup>a</sup>	Mean weight per bulb (grams) <sup>b</sup>
Fludioxonil	59.8 aA
Thiophanate methyl	57.1 aAbB
Benomyl	53.2 bBC
No fungicide	51.3 bC

<sup>a</sup> $n = 53$  for thiophanate methyl and benomyl, 54 for fludioxonil and control.

<sup>b</sup>Means followed by the same lower case letter do not differ at  $P \leq 0.05$ ; means followed by the same upper case letter do not differ at  $P \leq 0.10$ .

Table 3

Results of greenhouse trial, fungicides as preplanting dips for garlic cloves

Treatment <sup>a</sup>	Mean dry weight per plant (grams) <sup>b</sup>
Benomyl	16.09 a
Thiophanate methyl	11.23 b
Fludioxonil	9.96 b
No fungicide	9.73 b

<sup>a</sup> $n = 48$  plants per treatment, including dead plants receiving a score of zero.

<sup>b</sup>Means followed by the same letter do not differ at  $P \leq 0.05$  nor at  $P \leq 0.10$ .

Table 4

Results of field trial, fungicides as postharvest dips for garlic bulbs

Treatment <sup>a</sup>	Mean fraction of cloves rotted per bulb <sup>b</sup>
Benomyl	0.011 a
Thiophanate methyl	0.011 a
No fungicide	0.042 b

<sup>a</sup> $n = 52$  bulbs per treatment.

<sup>b</sup>Means followed by the same letter do not differ at  $P \leq 0.05$ .

Table 5

Results of laboratory dips of wounded, inoculated garlic bulbs

Treatment <sup>a</sup>	Mean fraction of cloves with lesions or blemish <sup>b</sup>
Benomyl	0.415 a
Thiophanate methyl	0.521 ab
No fungicide	0.790 b

<sup>a</sup> $n = 15$  bulbs per treatment.

<sup>b</sup>Means followed by the same letter do not differ at  $P \leq 0.05$ .

### Greenhouse trial of fungicides as preplanting dips

Treatment effects were significant ( $P \leq 0.006$ ), and replication and treatment interactions were not significant ( $0.71 > P > 0.14$ ), so replications were pooled for analysis. Means of live dry plant weights were greater than controls for all fungicide applications, but only the mean for benomyl was significant at  $P \leq 0.05$  or at  $P \leq 0.10$  (Table 3).

### Postharvest dips

Mean bulb weights for treatment with benomyl (40.7 g) or thiophanate methyl (40.9 g) failed to significantly differ from controls (38.6 g), nor were effects of row or interaction significant. Mean fraction of cloves rotted per bulb for treatments with benomyl (0.011) or thiophanate methyl (0.011) differed from control (0.042) at  $P = 0.02$ , with effects of row ( $P = 0.07$ ) and interaction ( $P = 0.14$ ) marginal. If rows are combined for analysis, the effects of benomyl and thiophanate methyl each differed from controls at  $P = 0.03$  (Table 4).

### Trials for eradication of pathogens in wounded and inoculated garlic bulbs

In the trial using *F. proliferatum*, benomyl performed superior to controls ( $P = 0.046$ ), but thiophanate

methyl did not ( $P = 0.144$ ) (Table 5). In the trial using *F. oxysporum* f. sp. *cepae*, neither fungicide yielded results differing materially from the control.

#### Bioassay for pathogens in field soil

A total of 12 *Fusarium* isolates were recovered from 38 onions grown in field soil. These 12 isolates comprised 3, 3, 3, 2 and 1 isolates corresponding to the five-sampled field locations, respectively. No *Fusarium* was observed growing from basal plate tissues excised from 16 plants in the control pots, although some saprophytes in other genera grew from such tissues in both treatments and controls. Of the 12 *Fusarium* isolates, 10 (representing four of the five field locations) were confirmed as pathogenic by production of lesions upon re-inoculation into onion, (controls inoculated with sterile, de-ionized H<sub>2</sub>O produced no lesions). One *Fusarium* isolate proved non-pathogenic (lesions did not differ from controls) and one was inconsistent in production of lesions. Representative pathogenic isolates were identified as *F. proliferatum* and *F.o.* f. sp. *cepae*.

#### Discussion

The presence of multiple pathogens in lots of seed garlic from six states and mainland China (Table 1) suggests that common pathogens of garlic are frequently transmitted within and between countries by germplasm sale and exchange. All of the pathogens detected in our survey or at the WRPIS Pullman farm (*Aspergillus niger*, *A. ochraceus*, *Botrytis porri*, *Embellisia alli*, *Fusarium oxysporum* f. sp. *cepae*, *F. proliferatum*, *F. verticillioides* and *P. hirsutum*) are widely distributed and probably cosmopolitan (see below). In addition to the pathogens in Table 1, some lots also contained the saprophytes *Aspergillus melleus* Yukawa, *A. sydowii* (Barn. & Sart.) Thom & Church, *A. versicolor* (Vuill.) Tiraboschi, *A. wentii* Wehmer, *Eurotium amstelodami* Mangin, *E. herbariorum* Link, *Penicillium chrysogenum* Thom, *P. citrinum* Thom, or *Scopulariopsis* sp.

Formal reporting from garlic appears lacking for *F. verticillioides*, but the fungus is widely distributed (Farr et al., n.d.). *Fusarium oxysporum* f. sp. *cepae* and *E. allii* are documented on garlic in Washington State (Dugan et al., 2006). Database documentation for *F. oxysporum* f. sp. *cepae* on garlic in the United States exists for California and Idaho (Farr et al., n.d.) and it is documented in other literature as affecting garlic worldwide (e.g., Havey, 1995); it has been recorded on onion from many Pacific Northwest (PNW) locales, including Washington, Idaho, British Columbia (Shaw, 1973) and Oregon (Thornton and Mohan, 1996). In the United States, analogous database records for *E. allii* appear confined to Louisiana, Oregon and Texas (Farr et al., n.d.; Glawe, n.d.). We have been unable to locate database reports of *E. allii* from Washington state, but *E. allii* is previously recorded from Oregon (Halliwell and MacSwan, 1962) and is known worldwide (Farr et al., n.d.). For the United States, database records for *P. hirsutum* (= *P. corymbiferum* Westling) on garlic exist only for California

(Farr et al., n.d.), but *P. hirsutum*, *F. oxysporum* f. sp. *cepae* and *E. allii* are recognized as occurring on garlic worldwide (David, 1991; Kozakiewicz, 1995; Brayford, 1996) and these fungi are well known to garlic industry pathologists (Fred Crowe, personal commun.). Records for *Botrytis porri* are scarcer, but it is reported from Australia, California, Nevada, Oregon, Quebec and Venezuela (Somerville et al., 1984; Cedeño et al., 2003; Farr et al., n.d.). In view of the widespread distribution of these fungi, we view survey results without alarm, but we caution that persons planting seed garlic may run the risk of introducing or amplifying these pathogenic species or more virulent isolates locally.

We note here that the correct name for the *Penicillium* pathogen of garlic remains a matter of some dispute. The name *P. corymbiferum* (= *P. verrucosum* var. *corymbiferum* (Westling) Samson, Stolk & Hadlok) was often used (e.g., Smalley and Hansen, 1962; Brammall, 1989), but has been largely replaced by the synonym *P. hirsutum* (Pitt, 2000). Brammall (1989) noted that various names have been applied to *Penicillium* species rotting garlic, and applied the name *P. hirsutum* to only a subset of his isolates, leaving the remainder simply as '*Penicillium* sp.'. Overy et al. (2005) found that amongst several isolates in their collection, only those identified as *Penicillium allii* Vincent & Pitt were strongly pathogenic to garlic, and that isolates bearing the name *P. hirsutum* were not very aggressive. Valdez et al. (2006) thought the name *P. allii* preferable to *P. hirsutum* for those isolates pathogenic to garlic in Argentina. However, some of our isolates produced deeply coloured exudates more characteristic of *P. hirsutum* than that of *P. allii* (Samson and Frisvad, 2004) and we have applied the name *P. hirsutum* to our isolates, which were very aggressive in artificial inoculations. We note that several species in section *Corymbifera*, including *P. allii*, were in the past treated as varieties of *P. hirsutum*, e.g. *P. hirsutum* var. *allii* (Vincent & Pitt) Frisvad (Frisvad and Filtenborg, 1989). Although the names *P. hirsutum* and *P. allii* have both been applied to isolates rotting garlic as indicated above (with synonyms of the former name long applied to *Penicillium* spp. rotting garlic), only type material of *P. allii* is from garlic. Type (neotype) material for *P. hirsutum* was actually isolated from aphids (Frisvad and Samson, 2004), thus resolution of the problem is not readily solved merely by consulting documentation for types.

*Fusarium proliferatum* has been demonstrated to produce fumonisin mycotoxins in market garlic in Germany (Seefelder et al., 2002). The pathogen is also a problem on onion in the PNW of the USA (Mohan et al., 1987; du Toit et al., 2003) and is reported on flowers of *Allium thunbergii* in Korea (Shin and Kim, 2001). Potential problems regarding mycotoxins on *Allium* produced in the PNW are not assessed. We are not aware of published reports of *F. verticillioides* on *Allium* spp. including garlic, but given earlier reports of *F. moniliforme* on *Allium* species (e.g. Blodgett,

1946; French, 1989) one cannot discount the possibility that taxa isolated from *Allium* and lumped under the epithet *moniliforme* included representatives of *F. verticillioides* in addition to *F. proliferatum*. Much has been reported on mycotoxin production by *F. verticillioides* (e.g., Shim and Woloshuk, 2001). Moreover, '*F. proliferatum* and *F. verticillioides* are the two most prolific producers of fumonisins identified to date,' and *F. proliferatum* produces several additional mycotoxins (Desjardins, 2006). We are relieved that our isolates of *Aspergillus ochraceus* and *A. niger*, many strains of which produce ochratoxin A (Klich, 2002), were not aggressive in our artificial inoculations. To our knowledge, *A. ochraceus* has not been reported from garlic in North America, but it is a common fungus reported from a wide range of soils, seeds, roots and other sources (Klich, 2002). Herrera (2005) list *A. ochraceus* (as its synonym, *A. alutaceus*) as a non-regulated organism causing aspergillus rot of garlic.

Response of garlic to basal rot has been documented by Rengwalska and Simon (1986), where all seven garlic accessions tested were to some degree susceptible to *F. oxysporum* f. sp. *cepae*, but with variation in response. The differences between PI540346#3 and FOC-8 in their production of symptoms on garlic, described above, demonstrate potential for analogous variation in aggressiveness of the pathogen. We have noticed degrees of aggressiveness in tests on onion as well. Breeding programs have successfully generated *Fusarium*-resistant germplasm for onion production (Cramer, 2000).

Bioassay of field soil demonstrated that soil cropped to garlic was infested with *F. proliferatum* and *F. o. f. sp. cepae*, both of which survived prolonged freezing temperatures. The latter fungus produces chlamydospores, and is well known for protracted survival in soil (Havey, 1995). *Fusarium proliferatum* does not produce chlamydospores (Nelson et al., 1983) but when associated with residue, this species was capable of surviving in soil over extended periods (Cotton and Munkvold, 1998). Because we recovered *F. verticillioides* in our survey of commercial seed garlic, we note that it is also documented as surviving well under field conditions; see Leslie and Summerell, 2006. We recovered no *E. allii* in our bioassay because *A. cepae* is not a normal host, but it is reported that the fungus can over-winter in plant debris or soil (David, 1991). *Botrytis porri* produces survival structures, sclerotia, of considerable size (Chilvers and du Toit, 2006). *Aspergillus ochraceus* can produce sclerotia (Klich, 2002). *Penicillium hirsutum*, however, does not persist for a long time in soil (Anon, 2004).

Although results of our miscellaneous fungicide trials sometimes attained significance (for fludioxonil and thiophanate methyl, Table 2; for benomyl, Tables 3 and 5; for benomyl and thiophanate methyl, Table 4), other experiments failed to demonstrate any effect. In experiments in which suspensions of conidia of *F. oxysporum* f. sp. *cepae*, *F. proliferatum* or *E. allii* had been injected several mm deep into the inner tissues of gar-

lic cloves, neither benomyl nor thiophanate methyl deterred subsequent rot, even with dip times so prolonged that clove skins became detached (data not shown). In the high disease pressure season of 2005–2006, preplanting dips of fludioxonil at label rates to over 2000 cloves failed to produce survival rates superior to controls (data not shown), although fludioxonil had previously been effective under conditions of less disease pressure (Table 2). In our experiments using bulbs superficially wounded and inoculated with either *F. proliferatum* or *F. oxysporum* f. sp. *cepae*, positive results at traditional significance levels were attained only with benomyl in the former (Table 5). Interestingly, in the absence of any prior wounding, subsequent rot in bulbs dipped in suspensions of *F. oxysporum* f. sp. *cepae* or *F. proliferatum* did not differ from controls (data not shown), confirming the result of Gargi and Roy (1988) that some form of predisposing injury is necessary in artificial inoculations. One plausible explanation for this variation in results is the degree to which pathogens may have penetrated tissues. As noted in the introduction, we have seen rot along the inner clove axis, but in other instances hard soil at lifting may have created more shallow infection courts. Attacks by bulb mites (*Rhizoglyphus* spp. and *Tyrophagus* spp.) and the wheat curl mite (*Eriophyes tulipae* Keifer) in storage can also promote rot (Covello et al., 2006). Aetiology of garlic rots is insufficiently explored, including the roles of quiescent (latent) infections such as documented for *Fusarium culmorum* by Crowe (1995).

There are alternatives to fungicides for promotion of plant health in seed garlic. Hot water treatments have been used against fungal, nematode and arthropod pests of garlic (Sims et al., 1976; Hannan and Sorensen, 2002). However, cultivars can markedly differ in temperature tolerance. Because most of our accessions contain insufficient amounts of germplasm for tolerance testing, we conducted no experiments with hot water treatments. Tissue culture has been routinely implemented to generate pathogen-free germplasm for commercial production in Australia (Zalberg, 2000) and California (M. Jenderek, personal commun.). Resources are not currently available to apply this method at WRPIS but several accessions are annually forwarded to the National Center for Genetic Resources Preservation at Ft. Collins, Colorado, for tissue culture and cryo-preservation. Tissue culture may ultimately assure germplasm free not only of fungi, but also viruses. Several viruses are known to infect WRPIS germplasm (Pappu et al., 2005). However, small- to medium-sized distributors of seed garlic will probably continue to lack access to tissue culture techniques.

Garlic has been grown since at least the beginning of the second millennium BC (Zohary and Hopf, 2000), obviously without benefit of modern plant protection technologies for the great majority of that time. It can be inferred that fungal infection of seed cloves will not always result in significant disease,

especially in the absence of conducive conditions. Why, for instance, has *A. ochraceus*, documented as pathogenic by Gargi and Roy (1988) and listed by Herrera (2005) as causing aspergillus rot, not been more of a problem in North America? However, germplasm is now exchanged more frequently and over greater distances than formerly, so plant protection technologies have heightened importance. Seed garlic can sometimes benefit from preplanting or postharvest fungicidal applications as demonstrated above (and we continue to use fungicides at WRPIS to maximize benefits to recipients of our germplasm), but our results also indicate there should be no assumption that application of the above fungicides at label rates will be eradicated or even consistently effective against fungal pathogens, especially under conditions of high disease pressure. We have embarked on a series of sustained trials with fungicides in order to define more precisely the degree and circumstances to which one or more fungicides may be cost-effective over long periods of use.

### Acknowledgements

Howard Schwartz for provision of FOC8. Gayle Volk and Martin Chilvers for helpful review of the manuscript, and Cerexagri and Syngenta for donation of fungicides. We thank John Fellman for instruction on proper construction and operation of the dip tanks. Further technical assistance was provided by Heather Clarkson, Russell Staska, Ellen Johnston, Alison Sigler, Leslie Elbersen and Wayne Olson.

### References

- Anon. (1925) Campanile (Guilla). *Rev Appl Mycol* **4**:325.
- Anon. (1980) Nakov et al. *Rev Plant Pathol* **59**:471.
- Anon. (2004) *Crop profile for garlic in California, Western IPM Center, CSREES-USDA*, <http://www.ipmcenters.org/CropProfiles/docs/CAgarlic.html>.
- Blodgett EC. (1946) Observations on blasting of onion seed heads in Idaho. *Plant Dis Rep* **30**:77–81.
- Brammall RA. (1989) Resistance to benomyl in isolates of *Penicillium* sp. causing clove decay of garlic. *Can J Plant Pathol* **11**:409–414.
- Brayford D. (1996) *Fusarium oxysporum* f. sp. *cepae*, IMI Descript. Fungi and Bacteria No. 1263. *Mycopathologia* **133**:39–40.
- Campanile G. (1924) Ricerche sopra le condizioni di attacco e di sviluppo di *Helminthosporium allii* su Aglio. *Staz Sperim Agrar Ital* **57**:413–428 + 4 figs.
- Cedeño L, Carrero C, Quintero K, Segovia P. (2003) *Botrytis porri*, causante de pudrición en el cuello del ajo en Mérida, Venezuela. *Interciencia* **28**:273–275.
- Centraalbureau voor Schimmelcultures. (n.d.) *Penicillium* Subgenus *Penicillium*: Polyphasic Identification. 10 October 2006, <http://www.cbs.knaw.nl/databases/index.htm>.
- Chilvers MI, du Toit LJ. (2006) Detection and identification of *Botrytis* species associated with neck rot, scape blight, and umbel blight of onion. *Plant Health Progress* DOI:10.1094/PHP-2006-1127-01-DG.
- Corlett M. (1996) Fungi Canadenses No. 336, *Embellisia allii*. *Can J Plant Pathol* **18**:486–487.
- Cotton TK, Munkvold GP. (1998) Survival of *Fusarium moniliforme*, *F. proliferatum* and *F. subglutinans* in maize stalk residue. *Phytopathology* **88**:550–555.
- Coviello RL, Chaney WE, Orloff S, Poole GJ, Davis RM, Aegerter BJ, Westerdahl BB, Becker JO, Smith R, Fennimore SA. *UC IPM Pest Management Guidelines: Onion and Garlic*. Oakland, California, UC ANR Publ. 3453, 2006, pp. 41.
- Cramer CS. (2000) Breeding and genetics of *Fusarium* basal rot resistance in onion. *Euphytica* **115**:159–166.
- Crowe FJ. *Fusarium* basal rot of garlic. In: Schwartz HF, Mohan SK (eds), *Compendium of Onion and Garlic Diseases*. St Paul, Minnesota, APS Press, 1995, pp. 11.
- Damicone J. (2002) Update on cancellation of benlate fungicide. *Plant Insect and Disease Advisory*, Oklahoma State University, May 14, 2002, [plants.okstate.edu/Pddl/advisory.htm](http://plants.okstate.edu/Pddl/advisory.htm).
- David JC. (1991) *Embellisia allii*. IMI Descr. Fungi and Bacteria No. 1078. *Mycopathologia* **116**:56–60.
- Desjardins AE. *Fusarium Mycotoxins: Chemistry, Genetics, and Biology*. St Paul, Minnesota, APS Press, 2006, pp. 260.
- Dugan FM, Hellier BC, Lupien SL. (2003) First report of *Fusarium proliferatum* causing rot of garlic bulbs in North America. *Plant Pathol* (web version: <http://www.bspp.org.uk/ndr/>).
- Dugan FM, Hellier BC, Lupien SL. (2006) Efficacy of fungicides against infections of garlic in Washington State. *Phytopathology* **96**:S167.
- du Toit LJ, Inglis DA, Pelter GQ. (2003) *Fusarium proliferatum* pathogenic on onion bulbs in Washington. *Plant Dis* **87**:750.
- Ellis MB. *Dematiaceous Hyphomycetes*. Kew, Surrey, England, IMI, 1971, pp. 608.
- Farr DF, Rossman AY, Palm ME, McCray EB. (n.d.) *Fungal Databases, Systematic Botany & Mycology Laboratory*. ARS, USDA. 10 October 2006, <http://nt.ars-grin.gov/fungal-databases/>.
- French AM. *California Plant Disease Host Index*. Sacramento, Calif Dept Food Agric, 1989, pp. 394.
- Frisvad JC, Filtenborg O. (1989) Terverticillate *Penicillia*: chemotaxonomy and mycotoxin production. *Mycologia* **81**:837–861.
- Frisvad JC, Samson RA. (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*: a guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. *Stud Mycol* **49**:1–173.
- Gargi, Roy AN. (1988) Prevention and control of some post harvest fungal diseases of garlic bulbs. *Pesticides (Bombay)* **22**:11–15.
- Glawe DA. (n.d.) *Pacific Northwest Fungi Database*. Department of Plant Pathology, Washington State University, Puyallup, Washington, 10 October 2007, <http://cru23.cahe.wsu.edu/fungi/programs/aboutDatabase.asp>.
- Halliwel RS, MacSwan IC. (1962) Undescribed plant diseases and new hosts recorded in Oregon during 1960–1961. *Plant Dis Rep* **46**:30.
- Hannan RM, Sorensen EJ. (2002) *Crop profile for garlic in Washington*. Washington State University Cooperative Extension MISC0369E, 14 pp.
- Havey MJ. *Fusarium* basal plate rot. In: Schwartz HF, Mohan SK (eds), *Compendium of Onion and Garlic Diseases*. St Paul, Minnesota, APS Press, 1995, pp. 10–11.
- Herrera V. *Import health standard commodity sub-class: fresh fruit/vegetables garlic, Allium sativum* from the People's Republic of China, DRAFT. New Zealand, Plant Protection Organization, 17 May 2005.
- Klich MA. *Identification of Common Aspergillus Species*. Utrecht, Centraalbureau voor Schimmelcultures, 2002, pp. 116.
- Kozakiewicz Z. (1995) *Penicillium hirsutum*: IMI Descr. Fungi and Bacteria No. 1260. *Mycopathologia* **132**:61–62.
- Leslie JF, Summerell BA. *The Fusarium Laboratory Manual*. Ames, Iowa, Blackwell, 2006, pp. 388.
- MacArthur M. (2001) Benlate fungicide to be phased out. *Western Producer*, 3 May 2001, <http://www.producer.com>.
- Mohan SK, Bijman VP, Knott EA. (1987) Bulb rot of onions caused by *Fusarium proliferatum*. *Phytopathology* **87**:S67.
- Nakov B, Boshnakov P, Angelov D. (1979) Sazdivi petna po ches"na I sredstva za borba. *Rastitelna Zashchita* **27**:22–25.
- Nan B. (1995) Fungicide seed treatments of sainfoin control seed-borne and root-invading fungi. *NZ J Agric Res* **38**:413–420.
- Nelson PE, Toussoun TA, Marasas WFO. *Fusarium Species: An Illustrated Manual for Identification*. University Park, Pennsylvania, Penn St Univ Press, 1983, 193 pp.
- Nirenberg H, O'Donnell K. (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90**:434–458.



- Overy DP, Frisvad JC, Steinmeier U, Thrane U. (2005) Clarification of the agents causing blue mold storage rot upon various flower and vegetable bulbs: implications for mycotoxin contamination. *Postharvest Biol Technol* **35**:217–221.
- Pappu HR, Hellier BC, Dugan FM. 2005. First report of *Onion yellow dwarf virus*, *Leek yellow stripe virus* and *Garlic common latent virus* in garlic in Washington State. *Plant Dis* **89**:205.
- Park C-S, Paulitz TC, Baker R. (1988) Biocontrol of *Fusarium wilt* of cucumber resulting from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium oxysporum*. *Phytopathology* **78**:190–194.
- Pitt JI. *A Laboratory Guide to Common Penicillium Species*. North Ryde, NSW, Australia, Food Science Australia, 2000pp. 197.
- Portela JA, Burba JL, Piccolo RJ. (1996) Control de carbonilla (*Helminthosporium allii* Camp.) en ajo (*Allium sativum* L.). I. Evaluación de métodos químicos y su efecto sobre la calidad. *Horticultura Argentina* **15**:38–45.
- Presly AH. (1985) Studies on *Botrytis* spp. occurring on onions (*Allium cepa*) and leeks (*Allium porrum*). *Plant Pathol* **34**:422–427.
- Rengwalska MM, Simon PW. (1986) Laboratory evaluation of pink root and *Fusarium* basal rot resistance in garlic. *Plant Dis* **70**:670–672.
- Samson RA, Hoeksma ES, Frisvad JC, Filtenborg O. *Introduction to Food- and Airborne Fungi*, 6th edn. Utrecht, Centraalbureau voor Schimmelcultures, 2000, pp. 389.
- Samson RA, Frisvad JC. (2004) *Penicillium* subgenus *Penicillium*: new taxonomic schemes and mycotoxins and other entolites. *Stud Mycol* **49**:1–260.
- Seefeldt W, Gossmann M, Humpf H-U. (2002) Analysis of fumonisin B<sub>1</sub> in *Fusarium proliferatum*-infected asparagus spears and garlic bulbs from Germany by liquid chromatography-electrospray ionization mass spectrometry. *J Agric Food Chem* **50**:2778–2781.
- Shaw GC. *Host Fungus Index for the Pacific Northwest – I. Hosts*. Washington State University, Pullman WA, Washington Agricultural Experiment Station Bulletin 765, 1973, 121 pp.
- Shim W-B, Woloshuk CP. (2001) Regulation of fumonisin B<sub>1</sub> biosynthesis and conidiation in *Fusarium verticillioides* by a cyclin-Like (C-Type) gene, *FCC1*. *Appl Environ Microbiol* **67**:1607–1612.
- Shin H-D, Kim J-W. (2001) Flower pink rot of *Allium* plants caused by *Fusarium proliferatum*. *Mycobiology* **29**:224–226.
- Simmons EG. (1971) *Helminthosporium allii* as the type of a new genus. *Mycologia* **63**:380–386.
- Simmons EG. (1983) An aggregation of *Embellisia* species. *Mycotaxon* **17**:216–241.
- Sims WL, Little TM, Voss RE. (1976) Growing garlic in California. University of California Cooperative Extension Leaflet 2948. 14 pp.
- Smalley EB, Hansen HN. (1962) Penicillium decay of garlic. *Phytopathology* **52**:666–678.
- Somerville PA, Hall DH, Greathead AS. (1984) Dry rot of garlic caused by *Botrytis porri* Buchw. *Phytopathology* **74**:829.
- Stevens R(ed) *Mycology Guidebook*. Seattle, University of Washington Press, 1981, 712 pp.665.
- Sumner DR. Black mold. In: Schwartz HF, Mohan SK. (eds), *Compendium of Onion and Garlic Diseases*. St Paul, Minnesota, APS Press, 1995, pp. 26–27.
- Swift CE, Wickliffe ER, Schwartz HF. (2002) Vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cepae* from onion in Colorado. *Plant Dis* **86**:606–610.
- Thompson WT. *Agricultural Chemicals. Book IV: Fungicides*. Fresno, California, Thompson Pub., 1991 pp. 198.
- Thornton MK, Mohan SK. (1996) Response of Spanish onion cultivars and numbered hybrids to basal rot and pink root. *Plant Dis* **80**:660–663.
- Valdez JG, Makuch MA, Ordovini AF, Masuelli RW, Overy DP, Piccolo RJ. (2006) First report of *Penicillium allii* as a field pathogen of garlic (*Allium sativum*). *Plant Pathol* **55**:583.
- Yohalem DS, Nielsen K, Nicolaisen M. (2003) Taxonomic and nomenclatural clarification of the onion neck rotting *Botrytis* species. *Mycotaxon* **80**:175–182.
- Zalcberg L. (2000) *Garlic – high yielding and virus-free for greater profits*. Australian Government, Rural Industries Research and Development Corporation, RIRDC Publ. No. 00/58.
- Zohary D, Hopf H. *Domestication of Plants in the Old World*, 3rd edn. Oxford, UK, Oxford University Press, 2000, pp. 316.